Control of seed storage product formation

Mature soybean seeds typically contain 35 to 50% protein, 15 to 25% lipid and about 10% nonstructural carbohydrate. Predominant seed storage proteins are the 11S glycinins and 7S β-conglycinins, which accumulate in membrane-bound protein bodies (Shewry et al. 1995). Lipids accumulate as triacylglycerols (TAGs) that are found in oil storage bodies surrounded by the protein oleosin or occasionally as oil droplets in the cytosol. Predominant fatty acids in TAGs are palmitate (16:0), stearate (18:0), oleate (18:1), linoleate (18:2) and linolenate (18:3). All of the biosynthetic steps leading to TAG synthesis are known (for review see Voelker and Kinney, 2001) and many of the genes encoding lipid biosynthetic enzymes have been identified (Mekhedove et al. 2000). In general, protein and oil content vary inversely and there is also an inverse relation between protein content and seed yield (Brim and Burton, 1979; Wilcox and Guodong, 1997). In order to manipulate seed composition and break the protein:yield barrier, it is essential to understand the biological mechanisms that control storage product formation in seeds. *This is one of the major long-term goals of this project.*

Soybean seeds develop in physical isolation from the maternal tissues. Assimilates (sucrose and amino acids) are obtained primarily from leaves (Rainbird et al. 1984) and are released from the seed coat into the apoplast prior to uptake by the developing cotyledons (Thorne 1980, 1981). Of particular interest to us is whether seed composition is controlled by supply of assimilates (sucrose and amino acids) or by intrinsic traits of the seed itself (e.g., inherent capacity for protein and/or oil biosynthesis). The literature provides evidence for both types of control. Briefly, evidence for assimilate (source) control comes from two types of studies. First, in reciprocal crosses of a limited number of genotypes, seed composition was less influenced by the genotype of the embryo than by the genotype of the plant on which the seeds developed (Singh and Hadley, 1968). Secondly, supply of supra-optimal N to a normal-protein soybean line resulted in seed protein contents approaching those of high-protein lines (Nakasanthien et al. 2000). These results indicate that N-availability to the developing seed is an important factor controlling storage protein synthesis and suggest that seeds of normal lines have intrinsic biochemical capacity to synthesize high protein concentrations if sufficient substrate is available.

However, control of seed composition by 'endogenous traits' is also suggested by the literature. In particular, using in vitro seed culture, Hayati et al. (1996) concluded that genotypic differences in seed protein are regulated by the cotyledons, not by N-supply. In addition, there are indications that seed composition is associated with factors such as seed P content (Bethlenfalvay et al. 1997), and the activity of certain enzymes such as phosphoenolpyruvate carboxylase (Sugimoto et al. 1989). Moreover, recent studies indicate that oil content of Arabidopsis seeds can be significantly increased by over-expression of diacylglycerol transferase (DGAT; Jako et al. 2001). Thus, the level of seed metabolites and the activities of key metabolic enzymes may also control protein:oil accumulation.

Our current working model for control of protein:oil synthesis is presented in simplified fashion in Figure 2. Assimilate supply from the maternal plant is clearly an important component, and we are speculating that in addition to the inherent capacity of the mother plant to supply assimilates from photosynthesis and N-assimilation, mechanisms may exist to keep the 'sink demand' of developing seeds in balance with provision of assimilates from 'source tissues.' It is well known that developing pods suppress cytokinin production in roots concurrent with inhibition of root growth (Noodén and Guiamét, 1989). The decrease in cytokinin production is required for monocarpic senescence of soybean (Noodén et al. 1990) and presumably the mobilization of reserves to developing seeds. Conceivably, the 'signals' that control root functions and leaf senescence are identical to those that coordinate sink demand with mobilization of source reserves during senescence. We will explore this level of control (identified as ① in Figure 2) in some novel genetic material we identified where this mechanism may not operate properly to

shut down assimilate supply when sink demand is low (low pod set in male sterile plants). Additional levels of control are postulated to involve transcriptional (②) and post-translational (③) mechanisms within the developing seed itself that mediate responses to changes in the availability of sucrose and amino acids. We are speculating that the metabolic priority of a developing soybean seed is to use available amide amino acids to form storage proteins. This will utilize some of the imported sucrose, as amide amino acid interconversions requires C-skeletons in the form of organic acids. Carbon skeletons derived from sucrose that are in excess of that needed for amino acid metabolism can be utilized for lipid biosynthesis (or accumulate as carbohydrate). It has been demonstrated with in vitro seed culture that as N-supply is increased, protein accumulation increased while oil accumulation decreased (Hayati et al. 1996). This inverse relationship between protein and oil could simply reflect that both pathways compete for C-skeletons derived from sucrose. However, we are speculating that N-metabolites (possibly Gln and/or Asn) may also regulate the expression of lipogenic mRNAs and thereby directly control the capacity for oil biosynthesis (shown in red in Figure 2). Furthermore, we are speculating (based on sequence analysis) that several key enzymes, including cytosolic pyruvate kinase (PK_C) and ω-6 desaturase may be regulated by protein phosphorylation, perhaps in response to metabolic signals. It is not yet clear what metabolites are transported into soybean plastids for fatty acid synthesis, but current evidence suggests that a plastid phosphoenolpyruvate (PEP)/phosphate antiporter (Fischer et al. 1997) may be providing the pyruvate required for fatty acid synthesis following metabolism of the imported PEP by plastidic pyruvate kinase (White et al. 2000). Thus, metabolism of PEP in the cytosol by cytosolic pyruvate kinase (to form pyruvate) and PEPcarboxylase (to form oxaloacetate) could supply the mitochondria with C-skeletons to form the organic acids required for amide amino acid interconversions and storage protein biosynthesis. Thus, expression of cytosolic pyruvate kinase, or modulation of activity by protein phosphorylation, could contribute to the control of sucrose utilization for protein versus oil biosynthesis.

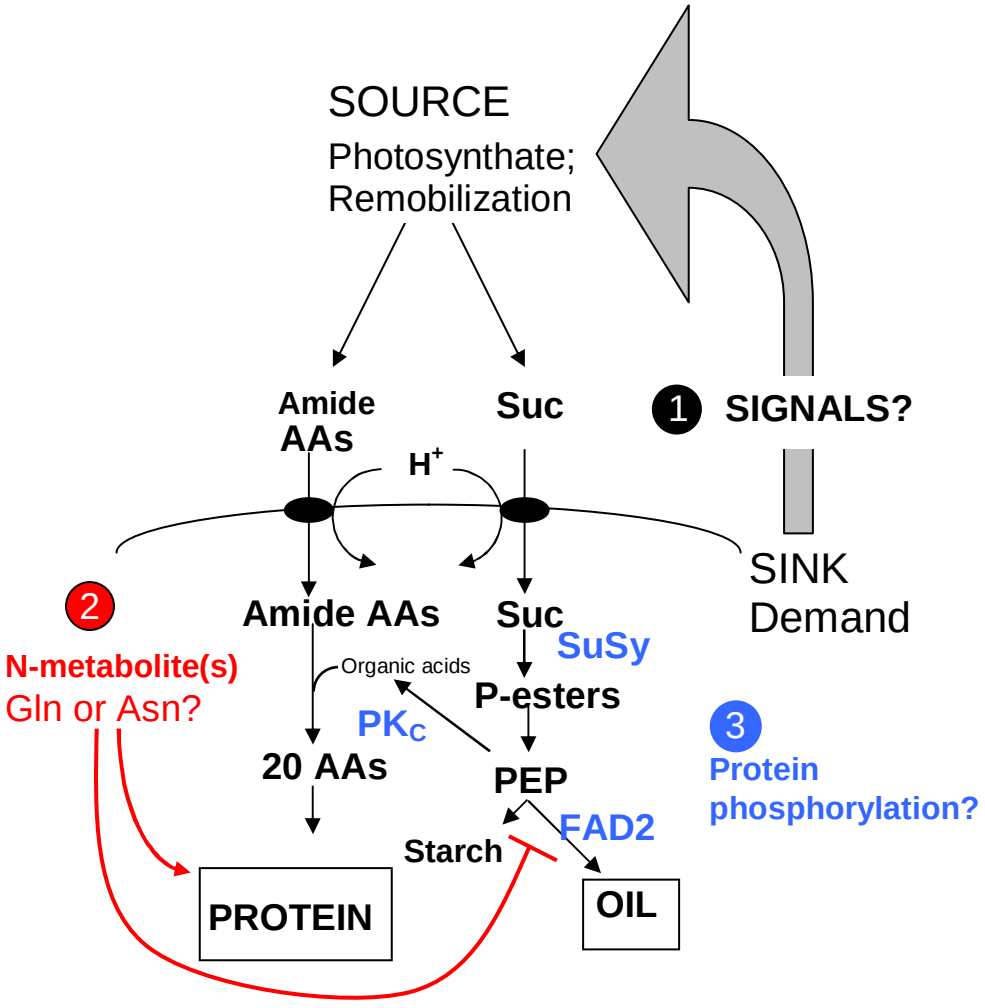

Figure 2. **Simplified schematic representation of three levels of control that may regulate metabolic reactions in a developing soybean seed leading to protein and oil biosynthesis (storage product formation).** ❶ involves signals that coordinate source supply of assimilates with sink demand; ❷ involves control by N-metabolites of storage protein synthesis (up regulation, known to occur) and oil biosynthesis (down regulation); and ❸ involves possible posttranslational control of key enzymes by reversible protein phosphorylation. Each of these will be explored in the proposed studies.